JACC Vol. 21, No. 1
January 1993:225–32

EXPERIMENTAL STUDIES

Passive Smoking Increases Experimental Atherosclerosis in Cholesterol-Fed Rabbits

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Objectives. We evaluated the influence of passive smoking on experimental atherosclerosis in cholesterol-fed rabbits.

Background. Exposure to environmental tobacco smoke (ETS) has been epidemiologically linked to death from ischemic heart disease in nonsmokers.

Methods. New Zealand male rabbits were randomly divided into three groups after 2 weeks of a 0.3% cholesterol diet. Sixteen rabbits were exposed to a high and 16 rabbits to a low dose of ETS; 32 rabbits located in another room served as an unexposed control group. After 10 weeks of ETS exposure, all rabbits were killed, and the percent of aortic and pulmonary artery endothelial surfaces covered by lipid lesions was measured by staining and planimetry.

Results. Average air nicotine, carbon monoxide and total particulate concentrations were 1,040 $\mu g/m^3$, 60.2 ppm and 32.8 mg/m^3 for the high dose ETS group, 30 $\mu g/m^3$, 18.8 ppm and 4.0 mg/m^3 for the low dose ETS group and <1 $\mu g/m^3$, 3.1 ppm and 0.13 mg/m^3 for the control group. The percent atherosclerotic involvement of the aorta and pulmonary artery increased significantly with ETS exposure (for the aorta, 30 ± 19% [mean ± SD] for the control group, 36 ± 14% for the low dose ETS group and 52 ± 21% for the high dose ETS group, $p < 0.001$; for the pulmonary artery, 22 ± 15% for the control group, 29 ± 25% for the low dose ETS group, and 45 ± 12% for the high dose ETS group, $p < 0.001$). Bleeding time was significantly shorter in the two ETS groups than in the control group (86 ± 17 vs. 68 ± 15, 68 ± 18 s, $p < 0.001$). There were no significant differences in serum triglycerides, cholesterol and high density lipoprotein cholesterol at the end of the study.

Conclusions. Environmental tobacco smoke affects platelet function and increases aortic and pulmonary artery atherosclerosis. This increase of atherosclerosis was independent of changes in serum lipids and exhibited a dose-response relation. These results are consistent with data from epidemiologic studies demonstrating that ETS increases the risk of death due to heart disease.

(J Am Coll Cardiol 1993;21:225–32)

Environmental tobacco smoke (ETS) is the term used to describe tobacco combustion products inhaled by nonsmokers in the proximity of burning tobacco. More than 4,000 constituents have been identified in cigarette smoke. Most exposure to ETS is from sidestream smoke emitted from the burning tip of the cigarette. Sidestream smoke is hazardous because it contains high concentrations of ammonia, benzene, nicotine, carbon monoxide and many other carcinogens and irritants (1–3).

Passive smoking involves breathing both sidestream smoke that goes directly into the air from the burning tobacco products and mainstream smoke after it has been exhaled by smokers. Sidestream smoke has higher concentrations of noxious compounds than does mainstream smoke. It has been estimated that approximately 50 million nonsmoking adults over the age of 35 years are regularly exposed to environmental tobacco smoke. Additionally, 50% of all children live in families with one or more smokers (4). The effects of passive smoking on health have been reported to include short-term effects, such as exacerbation of asthma and angina, as well as long-term effects, such as increased risk of lung cancer, respiratory tract infection and atherosclerosis (1–7).

Environmental tobacco smoke adversely affects platelet function and damages arterial endothelium, and depresses cellular respiration at the level of mitochondria (4,5). People exposed to it have significantly thicker arterial walls than do unexposed nonsmokers, and wall thickness is increased with increasing exposure (8). Passive smokers also have significantly depressed high density lipoprotein (HDL) cholesterol levels and significantly elevated ratios of total cholesterol to HDL cholesterol levels (9).

The materials in ETS may thus accelerate the development of atherosclerotic plaque. Previous experimental studies, however, showed that exposure to smoke from only 1 cigarette/day for 11 to 13 months failed to quantitatively affect atherosclerosis or serum lipids (10). We designed the present study to further evaluate the influence

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All editorial decisions for this article, including selection of referees, were made by a Guest Editor. This policy applies to all articles with authors from the University of California, San Francisco.

Manuscript received March 31, 1992; revised manuscript received July 7, 1992, accepted July 15, 1992.

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0735-1097/93/$6.00

JACC Vol. 21, No. 1
January 1993:225–32

of passive smoking on atherosclerosis in cholesterol-fed rabbits.

Methods

Protocol. Sixty-four New Zealand male rabbits (2.0 to 2.6 kg) were randomly separated into three groups and fed a high cholesterol diet for 12 weeks. The cholesterol diet (Ziegler Bros., Inc.) contained 3% soybean oil and 0.3% cholesterol by weight. The rabbits were housed in separate cages in well mixed exposure chambers (BioClean, Duo. Flo, model H 5500, Lab Products Inc.), 1.92 m × 1.92 m × 0.97 m (3.58 m^3), that accommodated eight rabbits in each group.

After 2 weeks on the diet, 16 rabbits, 8 at a time, were exposed to a high dose of sidestream smoke (high ETS group) from Marlboro filter cigarettes (4 cigarettes every 15 min for 6 h/day, 5 days/week) using a smoking machine (Heinr. Borgwald GMBH RM 1/G, D-2000 Hamburg, Germany) for 10 weeks from week 2 to week 12. Another 16 rabbits, 8 at a time, were given a low dose of smoke (low ETS group) from the same smoking machine through 20.5 feet of 10-mm inside diameter plastic tube attached to the mainstream port on the smoking machine. The smoke cooled and the large particles settled out in this tube, making the exposure level of the low ETS group similar to that of smoke spread by the ventilation system of a building from an area where smoke was permitted to nonsmoking areas of the same building. Thirty-two rabbits, 16 at a time, located in the same type of exposure chamber in another room but with no smoking machine, served as a control group eating the same diet for 12 weeks. Three fans in the exposure chambers were adjusted to ensure good mixing, using the measurement devices discussed later. At the end of the 6-h exposure period, the exhaust fan on the Bioclean unit was turned on and rapidly lowered the level of ETS pollution in the exposure chamber to background levels corresponding to those of the control animals until next day when the Bioclean unit was turned off and the smoking machine was turned on again.

Monitoring smoke exposure inside the chambers. We measured several constituents of ETS in the three exposure chambers: carbon monoxide (CO), total particulates, respirable suspended particulates and nicotine.

To measure average carbon monoxide concentrations during the 6-h exposure period, we used a model L15 CO Personal Exposure System (Langan Products) every other week for the three groups. We obtained an average daily value taken from 2,520 samples during the exposure period (3 h of ETS, 1 h break, 3 h of ETS) (Fig. 1).

To measure total particulate concentrations, we used a Miniram PDM-3 Optical Scattering Particle Monitor (MIE, Inc.), monitoring particulate concentration every 10 s, and computed average total particulate concentrations during the exposure period (Fig. 2). We obtained these data every other week for all three groups. We also used a Piezobalance

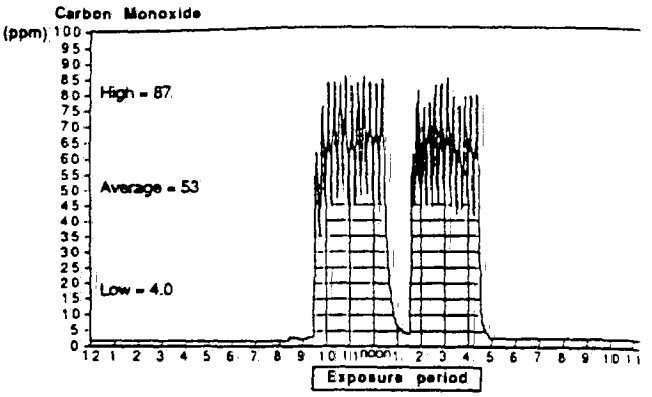


Figure 1. Representative carbon monoxide (CO) concentrations during a 24-h period. During the period of exposure to environmental tobacco smoke (ETS) (3 h of ETS, a 1-h break, 3 h of ETS), the average CO value from 2,520 samples is 53 ppm.

Respirable Aerosol Mass Monitor (model 3500 Thermo-System) to measure respirable suspended particulates (11) on 4 different days, about 10 samples/day, to calibrate the Miniram. The Piezobalance was factory calibrated before the study.

The Piezobalance measures the smaller respirable suspended particulates, whereas the Miniram measures total particulates. To determine the relation between particulate concentrations measured by the Miniram and Piezobalance, we measured average particulate concentration values (37 values, each an average of 3 measurements) at different levels of ETS using these two instruments simultaneously. Figure 3 shows that there was a strong linear relation between average particulate concentrations measured by the Piezobalance and the Miniram, with the Piezobalance reading about 36% of that obtained by the Miniram. This relation is similar to that found in a previous study (12) in which the Miniram and the Piezobalance were compared in an environmental chamber measuring ETS over a range of concentrations.

In addition, we monitored air nicotine levels by using a passive diffusion monitor (13) that was located in the middle of the exposure chamber during the 6-h exposure period, every other week for all three groups.

Hematologic and biochemical analysis. Bleeding time, circulating platelet aggregates, platelet count, hematocrit, hemoglobin, total serum cholesterol, triglycerides, high density lipoprotein (HDL) cholesterol and serum cotinine were measured at the beginning of the study (before the rabbits started the high cholesterol diet) and at 6-week intervals (that is, after 4 and 10 weeks). The concentration of cotinine was determined by gas chromatography with nitrogen-phosphorus detection (14). This method has been modified for simultaneous extraction of cotinine and determination using capillary gas chromatography (15).

Bleeding time was determined after 1-min warming of the rabbit's ear in a normal saline bath (37°C). A small standard

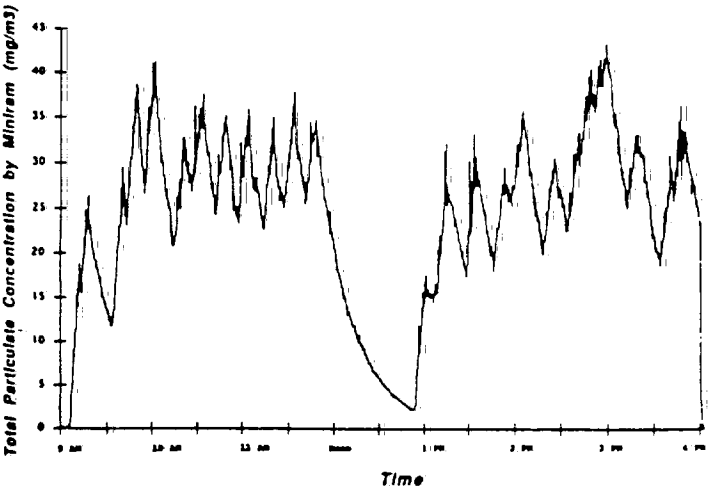
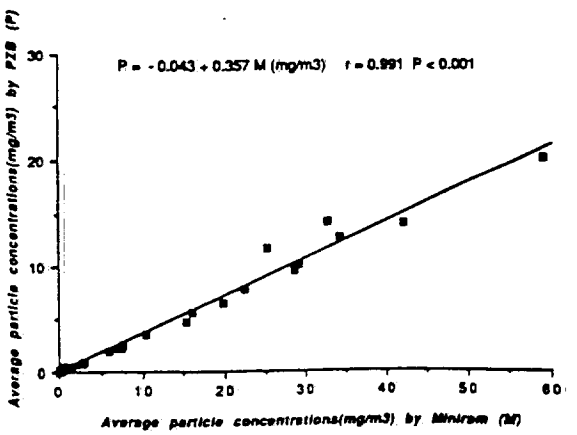


Figure 2. Total suspended particulate concentrations measured by the Miniram every 10 s during a representative period of exposure to environmental tobacco smoke (ETS) (3 h of ETS, a 1-h break, 3 h of ETS). Average total suspended particulate concentration during the exposure period was 23.7 mg/m^3. The peaks occur while the cigarettes are actually being smoked. The large drop corresponds to the 1-h midday break.

prick was made in the ear, avoiding macroscopic vessels. The time from the initial bleeding to cessation of bleeding was recorded as the bleeding time.

A platelet count ratio method (16) was used for quantitative determination of circulating platelet aggregates. One mmol/liter adenosine diphosphate was added to a citrated venous blood sample before stirring. The sample was divided into two tubes, one containing ethylenediaminetetraacetic acid (EDTA)/formalin solution and the other EDTA only. Platelet-rich plasma was collected after centrifugation. Platelets in both samples were counted, using standard techniques (Sequoia-Turner Corporation, Operator Reference manual, Cell-Dyn 900 Hematology Analyzer). The platelet aggregate ratio was calculated from the platelet count in the two solutions. The higher the ratio, the fewer the platelet aggregates.

Figure 3. Relation between average particulate concentrations measured by the Piezobalance (PZB) and the Miniram. Because of the excellent linear relation, one can measure respirable suspended particulates by taking 36% of the readings obtained with the Miniram.



Total serum cholesterol and triglyceride levels were determined by automated enzymatic methods (Coulter DART cholesterol reagent using the DACOS and DACOS XL analyzers), and HDL cholesterol concentrations were measured after precipitation of other lipoprotein classes with dextran and magnesium ions (HDL cholesterol precipitant (Cat No 236141), Ciba Corning Diagnostics Corp.).

The blood samples were drawn in the morning (Tuesday to Friday) after 12 h of fasting and before ETS exposure. The samples for plasma cotinine analysis also were taken in the morning before exposure (17 h after the last ETS exposure).

Morphologic studies. At week 12, after 10 weeks of exposure to ETS (or control conditions), all rabbits were killed. After intravenous administration of pentobarbital, 130 mg/kg body weight, the aorta was removed from its origin (2 cm distal to the aortic valve) down to the bifurcation of the internal iliac arteries; the pulmonary artery was isolated from its beginning at the pulmonary valve to just above the bifurcation. The vessels were opened by linear vertical incision, fixed in a 10% formalin solution for 24 h, stained with Sudan IV, then photographed. Finally, planimetric measurement of lipid lesions was performed quantitatively by estimating the total stained regions in photographs of each artery with a planimeter. The measurements were performed in blinded fashion and in duplicate.

Statistical analysis. The text and tables list data as the mean value ± SD; the figures summarize data as the mean value ± SEM. Data were analyzed by linear regression, using ETS dose as the independent variable. Multiple linear regression was also used with aortic and pulmonary artery lesions as the dependent variables, including cholesterol levels as well as exposure to smoke in the regression equation to account for the possible effects of different serum cholesterol levels on the extent of lesions. Analysis of variance (ANOVA) was used to compare observations among the three experimental groups. Data were analyzed before and after exposure, as well as in terms of changes in

Table 1. Average Air Nicotine, Carbon Monoxide and Particulate Concentrations in the Control and Environmental Tobacco Smoke Chambers

Group	Air Nicotine ($\mu g/m^3$)	Air CO (ppm)	Total Particulates* (mg/m^3)	Respirable Particulates† (mg/m^3)
Control	<1 (n = 1)	3.1 ± 1.9 (n = 2)	0.13 ± 0.04 (n = 10)	0.07 ± 0.06 (n = 10)
Low ETS	30 ± 3 (n = 4)	18.8 ± 2.2 (n = 5)	4.03 ± 0.49 (n = 3)	1.2 ± 0.7 (n = 8)
High ETS	1,040 ± 302 (n = 4)	60.2 ± 14.3 (n = 5)	32.8 ± 6.9 (n = 7)	13.8 ± 3.5 (n = 6)

*By Miniram. †By Piezobalance. Values are expressed as mean value ± SD. n = the number of samples. For nicotine, carbon monoxide [CO] and total particulates, each of the n samples represents the average value observed during the exposure period (3 h of environmental tobacco smoke [ETS], 1 h break, 3 h of ETS) on 1 day. For example, the n = 2 values for Air CO in the control group represent average values recorded during 7 h on 2 different days. For respirable particulates, the sample size represents the actual number of simple samples taken while the smoke levels were at steady state.

the measured variables before and after the 10-week exposure period using paired *t* tests. We did not combine all data into a single two-factor analysis of variance (with time [before or after exposure] as one factor and ETS group [control, low ETS, high ETS] as the second factor); we believed that with such an approach the presence of a control group (with no exposure) at both times would generally lead to a significant interaction between time and exposure group that would make the results of tests on the main effects difficult to interpret. Data were processed by using Minitab Versions 7.2 and 8.2. A p value < 0.05 was taken as statistically significant.

Results

Weight gain. There was a similar initial body weight and subsequent weight gain in all three groups of rabbits throughout the 12-week period. The average body weight before (week 2) and after 10 weeks of ETS exposure (week 12) was 2.7 ± 0.3 and 3.6 ± 0.3 kg, respectively. There was no significant difference in weight of the rabbits as assessed by ANOVA before ($p = 0.344$) or after ($p = 0.306$) the 12-week experimental period. Similarly, ANOVA showed no significant differences in weight gain among the three exposure groups (0.87 ± 0.29 kg for the control group, 0.88 ± 0.37 kg for the low ETS group and 0.91 ± 0.36 kg for the high ETS group, $p = 0.923$) or in food intake among the three groups, either before ($p = 0.398$) or after ($p = 0.431$) exposure to ETS. The average food intake before and after ETS exposure was 178 ± 46 and 164 ± 58 g/day, respectively. The similarities in eating and weight gain across time and the different exposure groups indicate that any differences observed in the exposure groups were not due to dietary differences. There were no deaths during the 12-week study.

Smoke exposure inside the chamber. The average air nicotine, carbon monoxide (CO) and total particulate concentrations during the 6-h exposure period are listed in Table 1. There were large differences in air nicotine, CO and particle concentrations between the groups with a high or low level of ETS exposure and the control group and between the high and low ETS groups during the period of exposure.

Alterations in lipids. After rabbits were fed a high lipid diet, the serum cholesterol increased considerably in all animals during the 12-week period. The serum lipid levels for the three groups (Table 2) show a similar increase in total serum cholesterol. Total cholesterol may have been slightly ($p = 0.051$) higher in the control group than in the two ETS groups before the 10-week exposure period. There was no significant difference ($p > 0.8$) among the three groups at the end of the experiment. There were no significant differences ($p > 0.3$) in triglycerides and HDL cholesterol among the two ETS groups and the control group either before or after the 10-week exposure period. There also were no significant differences ($p > 0.4$) in the area under the cholesterol time curve (cholesterol-weeks: 11,632 ± 3,479 vs. 9,831 ± 3,048 and 10,349 ± 3,182 mg/dl-wk), change (12-week value minus 2-week value) in cholesterol (538 ± 463 vs. 674 ± 419 and 729 ± 627, 674 ± 419 mg/dl), change in triglycerides (13 ± 84 vs. −63 ± 372 and 22 ± 91 mg/dl) and change in HDL cholesterol (15 ± 29 vs. 7 ± 22 and 16 ± 25 mg/dl),

Table 2. Effects of Environmental Tobacco Smoke on Serum Lipids in Cholesterol-Fed Rabbits

Group	Cholesterol (mg/dl)		Triglycerides (mg/dl)		HDL Cholesterol (mg/dl)	
	Before	After	Before	After	Before	After
Control (n = 32)	671 ± 278	1,209 ± 483	91 ± 72	78 ± 51	40 ± 16	55 ± 27
Low ETS (n = 16)	480 ± 279	1,154 ± 395	102 ± 93	165 ± 349	36 ± 13	43 ± 25
High ETS (n = 16)	531 ± 246	1,260 ± 532	119 ± 93	98 ± 111	37 ± 15	50 ± 21

Values are expressed as mean value ± SD. There were no significant differences ($p > 0.3$) among the three groups except for total cholesterol before exposure to environmental tobacco smoke (ETS). Values in the control group were higher than values in the other two groups ($p = 0.05$). After = 12 weeks on lipid diet and 10 weeks of smoke exposure; Before = 2 weeks on lipid diet and before smoke exposure; HDL = high density lipoprotein.

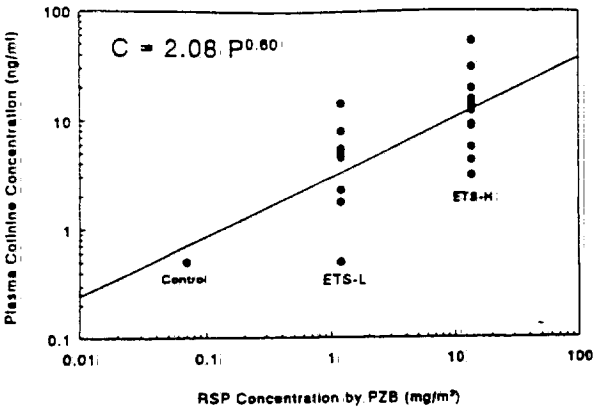


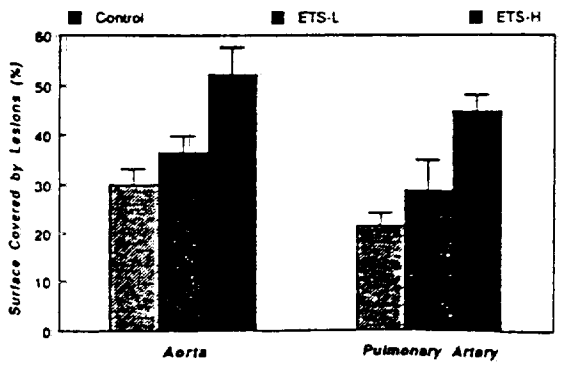
Figure 4. Relation between log-plasma cotinine levels and log-respirable suspended particulates (RSP) concentrations measured by Piezobalance (PZB). ETS-H and ETS-L = groups with a high or low level, respectively, of exposure to environmental tobacco smoke.

respectively, among the control group and the low and high ETS groups.

Cotinine levels in plasma. The plasma cotinine levels at the 6th week of ETS exposure in the control and the low and high ETS groups were <1.0, 6.0 ± 4.3 and 15.6 ± 12.3 ng/ml, respectively. These cotinine levels are based on blood samples drawn in the morning before that day's exposure to ETS. Given the 20-h half-life of cotinine in the blood, the steady state cotinine levels at the end of the daily exposure period would be approximately <1, 12 and 31.2 ng/ml, respectively, for the control and low and high ETS groups. There was a linear relation between log cotinine levels in plasma and log average respirable suspended particulate concentrations measured by Piezobalance ($r = 0.84$, $p < 0.001$) (Fig. 4).

Morphologic studies. Figure 5 shows the percentage of total aortic and pulmonary artery surface area covered by

Figure 5. Percent of aortic and pulmonary artery surface areas covered by atherosclerotic lesions for each group. There is a significant ($p < 0.001$) dose-response relation for both vessels. Error bars are SEM. Abbreviations as in Figure 4.



lipid lesions in the three experimental groups. There was a significant ($p < 0.001$) dose-response relation for the extent of lipid lesions for both the aorta and the pulmonary artery as a function of respirable suspended particulate concentration measured by Piezobalance. Although the intercepts of the dose-response relations for the two arteries are significantly different (31.3 ± 2.7% for the aorta vs. 23.0 ± 2.6% for the pulmonary artery, $p < 0.05$), the slopes are not (1.62 ± 0.41%/mg/m^3 vs. 1.69 ± 0.39%/mg/m^3, $p > 0.5$). These results indicate that, although the baseline levels of lipid deposits in these two arteries are different, the effects of exposure to ETS on the two arteries are similar in terms of increased lipid deposits. There were also positive correlations ($r = 0.5$, $p < 0.001$) between the percent of lipid lesions in both arteries and the average CO levels. As with the relation between lesions and particulate concentration, the aorta initially had more lipid deposits than did the pulmonary artery, but both vessels showed similar increases (~0.5%/ppm) in lipid deposits with ETS exposure as CO was increased. Because particulate and CO levels are highly correlated, we cannot say whether either or both (or other) elements of the ETS are responsible for the dose-dependent increase in lipid deposits we observed. We can conclude unequivocally that there were significant ($p < 0.001$) dose-dependent increases in lipid deposits on both vessels with increasing ETS exposure.

Platelet function. Data on bleeding time, platelet aggregate ratio and platelet count are shown in Table 3. Bleeding times at week 12 in the low and high ETS groups were significantly shorter than those in the control group (68 ± 15, 68 ± 18 vs. 86 ± 17 s, respectively, $p < 0.001$). This result demonstrates that there were large (20%) changes in bleeding time at low levels of exposure to ETS and that further increases in exposure did not produce an additional effect. The platelet aggregate ratio at week 12 in the high ETS group may have been lower than the control level (79.4 ± 10.7 vs. 88.0 ± 12.2%, $p = 0.07$ by paired t test), reflecting an increase in platelet aggregates in the high ETS group. The platelet counts were modestly decreased to a similar extent in all three groups (Table 3). The changes in platelet count before and after exposure were −36 ± 97, −84 ± 131 and −94 ± 95 ($p = 0.151$ by ANOVA), respectively. These data show effects on platelet function at low levels of ETS that do not increase with further increases in dose. This result suggests that platelets are sensitive to low levels of ETS, after which the effect is saturated.

Discussion

Active smoking has consistently been identified as a major risk factor for ischemic heart disease. Exposure to environmental tobacco smoke (ETS), as passive smoking, has now been linked to heart disease in nonsmokers (4,6,17–19). Epidemiologic studies conducted in a variety of locations reflect about a 30% increase in risk of death from ischemic heart disease or myocardial infarction among non-

Table 3. Effects of Environmental Tobacco Smoke on Platelet Function in Cholesterol-Fed Rabbits

Group	Bleeding Time (s)		Platelet Aggregation (%)		Platelet Count (10^3)	
	Before	After	Before	After	Before	After
Control (n = 32)	78 ± 23	86 ± 17	84.1 ± 14.6	80.9 ± 13.7	295 ± 89	256 ± 89
Low ETS (n = 16)	73 ± 26	68 ± 15	83.9 ± 11.8	82.6 ± 14.3	352 ± 130	268 ± 95
High ETS (n = 16)	77 ± 19	68 ± 18	87.9 ± 12.3	79.4 ± 10.7*	372 ± 140	293 ± 76

*$p < 0.01$ compared with values in the control group. †$p = 0.07$, compared with values in the high ETS group before exposure. Values are expressed as mean value ± SD. Abbreviations as in Table 2.

smokers living with smokers (4–6,18). The larger studies also demonstrate a significant dose-response effect, with greater exposure to ETS associated with a greater risk of death from heart disease.

Our present study shows that passive smoking significantly increases aortic and pulmonary artery atherosclerosis in cholesterol-fed rabbits in a dose-dependent manner. There was a strong positive correlation between the percent of atherosclerotic lesions and the average CO or particulate concentrations, with the lipid deposits in arteries in the high dose group nearly doubling in just 10 weeks. These results are consistent with epidemiologic studies demonstrating that ETS increases the risk of death from heart disease.

Passive smoking and atherosclerosis. Smoking has long been recognized as one of the major risk factors for adult coronary heart disease, peripheral arterial disease, abdominal aortic aneurysm and stroke. Clinical investigations indicated that the proportion of intimal surface involved with atherosclerotic lesions in both the aorta and the right coronary artery was positively associated with serum very low density lipoprotein and low density lipoprotein cholesterol and was negatively associated with serum HDL cholesterol. The serum thiocyanate concentration, a marker for smoking, was strongly associated with the prevalence of atherosclerotic lesions, particularly in the abdominal aorta (20). Population studies of passive smokers revealed that passive smokers had significantly thicker carotid arterial walls than those of persons who had never smoked passively or actively (8). Our results are consistent with what one would expect from these clinical studies.

However, we observed much larger effects of ETS than would be expected from a simple dose-based extrapolation from high doses experienced by smokers to the lower doses of smoke experienced by nonsmokers. Our results suggest that nonsmokers may be more sensitive to the toxins in ETS than smokers are, perhaps because smokers have somehow adapted to the chronic poisoning associated with active smoking. It is also probable that some of the biochemical systems involved are very sensitive to ETS but saturate at low doses.

Passive smoking and serum lipids. Epidemiologic studies have suggested that there is a dose-response relation between the number of cigarettes smoked/day and increasing levels of plasma cholesterol (21). The HDL cholesterol level was lower in children exposed to ETS; the HDL_2 subfraction was reduced in boys, whereas the HDL_3 subfraction was reduced in girls. As a result, exposure of children to ETS may increase the risk of premature coronary heart disease (22). Nonsmoking adolescents with two smoking parents had significantly higher plasma cotinine concentrations after an adjustment for other factors than did adolescents whose parents did not smoke. A plasma cotinine concentration >2.5 μg/ml was associated with an 8.9% greater ratio of total cholesterol to HDL cholesterol and a 6.8% lower HDL cholesterol level (23). Similar results have been reported for nonsmoking adults exposed to ETS in the workplace (9). These results suggest that passive smoking, like active smoking, leads to alterations in lipid profiles predictive of an increased risk of atherosclerosis.

The present study, however, showed no significant differences in total serum cholesterol, triglycerides, HDL-cholesterol, cholesterol-weeks, change in cholesterol, change in triglycerides or change in HDL cholesterol between the control group and the two passive smoking groups.

To test whether the changes in lipid lesions associated with ETS exposure could be a result of differences in cholesterol levels, we performed a multiple regression analysis with the percent aorta and pulmonary artery with lipid deposits as the dependent variables and cholesterol, triglycerides and HDL cholesterol levels at 2 and 12 weeks (that is, before and after ETS exposure control) and ETS concentration as the independent variables. In both cases ETS exposure was still significant ($p < 0.001$) and positively associated with ETS dose after accounting for differences in serum cholesterol. Therefore, the increase in atherosclerotic lesions in the cholesterol-fed rabbits exposed to ETS was independent of changes in serum lipids in the present study.

Passive smoking and platelet function. In addition to their role in acute thrombus formation, platelets have also been implicated in the development of atherosclerosis. Davis et al. (17) reported that mean values of the platelet aggregate ratio before and after passive smoking were 0.87 and 0.78, respectively ($p = 0.002$). These values are similar to those we observed in the high ETS group (Table 3). They found that passive smoking increased platelet aggregation with a magnitude similar to that observed in active smoking. The effects of cigarette smoking on the levels of platelet-activating factor (PAF), one of the most potent proinflammatory agents, or PAF-like lipids were studied (24,25). The

results showed that the values of PAF-like lipids in both LDL and HDL in smokers increased significantly after smoking, and that the activity of plasma enzyme, PAF acetylhydrolase, was inhibited by cigarette smoke extract in a dose-dependent manner. The charge alteration reactions and PAF-acetylhydrolase inhibition appear to be localized at different sites on the lipoprotein molecule. Thus, the observed inhibition may account for the increase in plasma PAF concentration that is known to occur in smokers.

In the present study, bleeding times at week 12 in the two ETS groups were significantly shorter than in the control group ($p < 0.001$), and platelet aggregate ratio at week 12 in the high ETS group was borderline lower than the control level ($p = 0.07$), suggesting increased platelet aggregate formation. These results suggest that the effects of passive smoking may be mediated, at least in part, by altered platelet function.

Passive smoking and arterial endothelium. People exposed to ETS had a significantly thicker carotid artery wall than that of nonexposed persons who had never smoked, with the increase in wall thickness increasing with greater ETS exposure (8). Such epidemiologic studies are complemented by a variety of physiologic and biochemical data showing that ETS damages arterial endothelium. Davis et al. (17) reported that mean values of anuclear endothelial cell carcasses in venous blood before and after passive smoking were 2.8 and 3.7 ($p = 0.002$). The appearance of these cell carcasses indicates damage to the endothelium, which is the initiating step in the atherosclerotic process. Bondjers et al. (26) hypothesized that the effect of smoking might be mediated by increased catecholamine levels. The endothelial injury induced by smoking could be inhibited by metoprolol, supporting this hypothesis.

Other possible mechanisms of atherogenesis induced by ETS. Clinical studies (27–29) in smokers with coronary artery disease show that smoking increases myocardial oxygen demands and such indicators as the rate-pressure product). Also, smoking-induced coronary vasoconstriction, which is due to an alpha-adrenergically mediated increase in coronary artery tone, is prevented by calcium antagonist drugs and nitroglycerin. Thus, smoking can adversely affect the balance between myocardial oxygen supply and demand.

Several animal studies (5) have also shown that injections of polycyclic aromatic hydrocarbons, in particular 7,12-dimethylbenz(a,h) anthracene and benzo(a)pyrene, significantly increase aortic plaque and accelerate the development of atherosclerosis. These studies provide evidence that known carcinogenic chemicals can be atherogenic as well. In animal experiments, ETS also depresses cellular respiration at the level of mitochondria (30). The effects of ETS on cardiovascular function, platelet function, neutrophil function and plaque formation are the probable mechanisms leading to heart disease (4,5).

Dose and duration. In the present study, the average concentrations of air nicotine, CO and particles during 7 h of exposure in the high ETS group were 30-fold, 3-fold and 10-fold higher than in the low ETS group (1,040 vs. 30 $\mu g/m^3$, 60 vs. 19 ppm, 13.8 vs. 1.2 mg/m^3, respectively). Human exposure studies (5,11,13) showed that nicotine and respirable suspended particulate levels in restaurants ranged from 1 to 25 $\mu g/m^3$ and 55 to 600 $\mu g/m^3$, respectively; respirable suspended particulate levels were 589 to 1,140 $\mu g/m^3$ in bars and bingo halls (3). The U.S. National Ambient Air Quality Standard for respirable particles is 50 $\mu g/m^3$ (annual average). The nicotine levels in smoking sections on airplanes were found to be 50 to 100 $\mu g/m^3$. Air nicotine, CO and respirable suspended particulate levels in some public smoking rooms were found to range from 50 to 500 $\mu g/m^3$, 5 to 50 ppm and 0.50–1.95 mg/m^3, respectively. Thus, the levels we observed in the high ETS group are a factor of 2 to 10 higher than those observed in routine human environments and the levels in the low ETS group are similar to those of heavily smoking-polluted, but realistic, human environments.

The studies (31) reviewed show that cotinine measurements are sensitive to the current exposure of nonsmokers to other people's tobacco smoke with a half-life of ≈20 h in the blood. Plasma cotinine levels after 2 h of exposure to ETS in a heavily polluted public house were 7.33 ng/ml. The cotinine levels in plasma we observed in the low ETS group were comparable to those of a heavily polluted room, whereas those in the high ETS group were two- to fourfold higher.

Despite exposure to higher than routine human exposure levels, every rabbit in the two ETS groups tolerated the exposure well during the 10-week exposure period. There were no differences in food consumption or weight gain among the different experimental groups. The differences between these experimental exposure levels and actual human exposure levels were small compared with those of other studies of environmental toxins, where extrapolations >5 to 6 orders of magnitude are common. Indeed, our low ETS group represented realistic exposure for people who work in smoking environments, such as bartenders or waiters working in the smoking section of a restaurant.

Conclusions. These data indicate that the exposure of lipid-fed rabbits to passive smoke adversely affects platelet function and significantly increases atherosclerotic lesions in the aorta and pulmonary artery. This increase in atherosclerosis is independent of changes in serum lipids and has a dose-response relation. These results are consistent with epidemiologic studies demonstrating that ETS increases the risk of death from heart disease.

Grateful appreciation is given to James Repace of the Environmental Protection Agency, Washington, DC, Wayne Ott of EPA and Stanford University, Department of Statistics, Stanford, California and Lee Langan of Langan Products, Inc. for their many thoughtful suggestions. We acknowledge John Hudson of the Electronic Facilities, Cardiovascular Research Institute, University of California, San Francisco, San Francisco, California for technical assistance, and Paul E. Sumner, San Francisco for invaluable assistance in the platelet aggregation studies. We also thank S. Katharine Hammond of the

University of Massachusetts, Medical School, Worcester, Massachusetts for help in analyzing the air nicotine levels.

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